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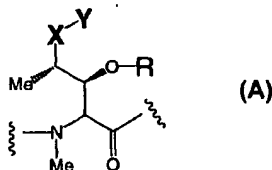
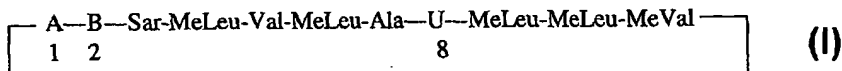
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[Continued on next page]

(54) Title: CYCLOSPORINS FOR THE TREATMENT OF IMMUNE DISORDERS



(57) Abstract: The present invention provides a cyclosporin of the following Formula (I), A compound of Formula (I) or a pharmaceutically acceptable salt, ester, or prodrug thereof: wherein: A is (A) X is selected from the group consisting of: $-(CH_2)_n-$ and $-CH_2-CH=CH-(CH_2)_m-$, where n is an integer of from 2 to 8 and m is an integer of from 2 to 5; Y is selected from the group consisting of: deuterium; halogen SCN; NCO; NCS; NO₂; OR₁, where R₁ is selected from the group consisting of: C₁-C₆ alkyl optionally substituted with aryl, substituted aryl, heteroaryl, substituted heteroaryl, halogen, C₃-C₆ alkenyl optionally substituted with aryl, substituted aryl, heteroaryl, substituted heteroaryl, halogen, C₃-C₆ alkynyl optionally substituted with aryl, substituted aryl, heteroaryl, substituted heteroaryl, halogen, aryl, substituted aryl, heteroaryl, and substituted heteroaryl; OC(O)-W-R₁, wherein W is absent, -O-, or -NH-, and R₁ is as previously defined; OCH₂SR₂, wherein R₂ is C₁-C₆ alkyl optionally substituted with aryl, substituted aryl, heteroaryl, substituted heteroaryl; SR₃, wherein R₃ is selected from the group consisting of: hydrogen, thiol protecting group, and R₁ as previously defined; and S(O)_nR₁, where n is an integer 1 or 2 and R₁ is as previously defined; R is selected from the group consisting of: hydrogen and a hydroxyl protecting group; B is selected from the group consisting of: -αAbu-, -Val-, Thr- and -Nva-; and U is selected from the group consisting of: -(D)Ala-, -(D)Ser-, -[O-(2 hydroxyethyl)(D)Ser]-, -[O-acyl(D)Ser]- and -[O-(2-acyloxyethyl)(D)Ser]-.



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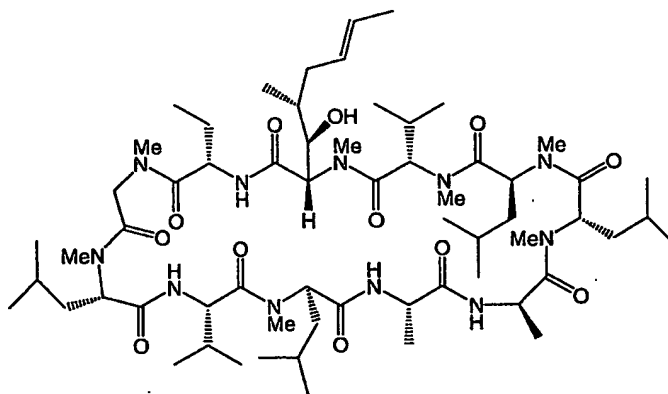
CYCLOSPORINS FOR THE TREATMENT OF IMMUNE DISORDERS

Technical Field

The present invention relates to novel semisynthetic cyclosporin analogs for the prevention of organ transplantation rejection and the treatment of immune disorders and inflammation, their use as pharmaceuticals and pharmaceutical compositions comprising them, as well as the processes for their production.

Background of the Invention

The cyclosporins comprise a class of structurally distinctive, cyclic, poly-N-methylated undecapeptides, commonly possessing pharmacological, in particular immunosuppressive, anti-inflammatory and antiparasitic activity. The first of the cyclosporins to be isolated was the naturally occurring fungal metabolite Cyclosporin or Cyclosporin, also known as cyclosporin A.



Cyclosporin A

MeBmt-αAbu-Sar-MeLeu-Val-MeLeu-Ala-DAla-MeLeu-MeLeu-MeVal										
1	2	3				8				11

Since the original discovery of Cyclosporin, a wide variety of naturally occurring cyclosporins have been isolated and identified, and many further non-natural cyclosporins have been prepared by total- or semi-synthetic means or by the application of modified culture techniques. The class comprised by the

cyclosporins is thus now substantial and includes, for example, the naturally occurring cyclosporins A through Z [cf., Traber et al.;1, *Helv. Chim. Acta*, 60, 1247-1255 (1977); Traber et al.; 2, *Helv. Chim. Acta*, 65, 1655-1667 (1982); Kobel et al.; *Europ. J. Applied Microbiology and Biotechnology*, 14, 273-240 (1982); and von
5 Wartburg et al.; *Progress in Allergy*, 38, 28-45 (1986)], as well as various non-natural cyclosporin derivatives and artificial or synthetic cyclosporin derivatives and artificial or synthetic cyclosporins including dihydrocyclosporins [in which the the –MeBmt-residue is saturated by hydrogenation]; derivatized cyclosporins (e.g., in which the 3'-O-atom of the –MeBmt- residue is acylated or a further substituent is
10 introduced at the α -carbon atom of the sarcosyl residue at the 3-position); and cyclosporins in which variant amino acids are incorporated at specific positions within the peptide sequence, e.g. employing the total synthetic method for the production of cyclosporins developed by R. Wenger—see e.g. Traber et al., 1; Traber et al., 2; and Kobel et al., loc cit. U.S. Pat. Nos. 4,108,985, 4,220,641,
15 4,288,431, 4,554,351, 4,396,542 and 4,798,823; European Patent Publication Nos. 34,567A, 56,782A, 300,784A and 300,785; International Patent Publication No. WO 86/02080 and UK Patent Publication Nos. 2,206,119 and 2,207,678; Wenger 1, *Transpl. Proc.*, 15 Suppl. 1:2230 (1983); Wenger 2, *Angew. Chem. Int. Ed.* 24 77 (1985) and Wenger 3, *Progress in the Chemistry of Organic Natural*
20 *Products*, 50, 123 (1986).

The compound cyclosporine (cyclosporine A or CsA) has found wide use since its introduction in the fields of organ transplantation and immunomodulation, and has brought about a significant increase in the success rate for transplantation procedures. Undesired side effects associated with cyclosporine, however, such
25 as nephrotoxicity, have led to a continued search for immunosuppressant compounds having improved, efficacy and safety.

Side effects with systemic CsA include increase in diastolic blood pressure and decrease in renal function. Other side effects include hepatic dysfunction, hypertrichosis, tremor, gingival hyperplasia and paraesthesia. The systemic toxicity
30 of CsA limits its use for the treatment of certain diseases.

Summary of the Invention

The present invention relates to novel cyclosporin analogs and methods of treatment for the prevention of organ transplantation rejection and the treatment of immune disorders or inflammation in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production.

More particularly, the present invention provides a cyclosporin of the following Formula (I),

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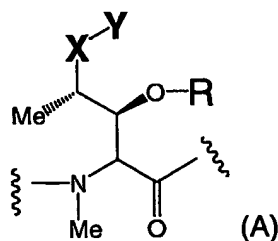


(I)

or a pharmaceutically acceptable salt, ester or prodrug thereof.

15

In Formula (I), A is



X is selected from the group consisting of: $-(CH_2)_n-$ and $-CH_2-CH=CH-(CH_2)_m-$, where n is an integer of from 2 to 8 and m is an integer of from 2 to 5;

Y is selected from the group consisting of:

deuterium;

halogen;

SCN;

25

NCO;

NCS;

OR₁, where R₁ is selected from the group consisting of:

hydrogen,

C₁-C₆ alkyl optionally substituted with aryl, substituted aryl, heteroaryl,

30

substituted heteroaryl, halogen,

C₃-C₆ alkenyl optionally substituted with aryl, substituted aryl, heteroaryl,
 substituted heteroaryl, halogen,
 C₃-C₆ alkynyl optionally substituted with aryl, substituted aryl, heteroaryl,
 substituted heteroaryl, halogen,
 5 aryl,
 substituted aryl,
 heteroaryl, and
 substituted heteroaryl;
 OC(O)-W-R₁, wherein W is absent, -O-, or -NH-, and R₁ is as previously
 10 defined;
 OCH₂SR₂, wherein R₂ is C₁-C₆ alkyl optionally substituted with aryl,
 substituted aryl, heteroaryl, substituted heteroaryl;
 NHR₁, wherein R₁ is as previously defined;
 N(R₁₀)R₁, wherein R₁ is as previously defined and R₁₀ is selected from the
 15 group consisting of: hydrogen, -CH₃, and -CH₂CH₃;
 SR₃, wherein R₃ is selected from the group consisting of: hydrogen, thiol
 protecting group, and R₁ as previously defined; and
 S(O)_nR₁, where n is an integer 1 or 2 and R₁ is as previously defined;
 R is selected from the group consisting of: hydrogen and a hydroxyl protecting
 20 group;
 B is selected from the group consisting of: -αAbu-, -Val-, -Thr- and -Nva-; and
 U is selected from the group consisting of: -(D)Ala-, -(D)Ser-, -[O-(2
 hydroxyethyl)(D)Ser]-, -[O-acyl(D)Ser]- and -[O-(2-acyloxyethyl)(D)Ser]-.

25 In Formula (I), amino acid residues referred to by abbreviation, eg. -Ala-, -
 MeLeu-, -αAbu-, etc., are, in accordance with conventional practice, to be
 understood as having the L-configuration unless otherwise indicated. (For
 example, -(D)Ala- represents a residue having the D-configuration). Residue
 abbreviation preceded by "Me" as in the case of "MeLeu", represents an α-N-
 30 methylated residue. Individual residues of the cyclosporin molecule are numbered,
 as in the art, clockwise and starting with the residue, -MeBmt- corresponding to
 residue 1. The same numerical sequence is employed throughout the present
 specifications and claims.

Accordingly, the present invention provides the use of cyclosporin analogs for the manufacture of a preparation for the treatment, with or without the concurrent use of other drugs, of organ transplantation rejections, immune disorders, and inflammation including rheumatoid arthritis, psoriasis, inflammatory bowel diseases, chronic obstructive pulmonary disease, allergic rhinitis, and asthma.

Detailed Description of the Invention

10

A first embodiment of the invention is a compound represented by Formula I as described above, or a pharmaceutically acceptable salt, ester or prodrug thereof.

Representative compounds of the invention include, but are not limited to, the compounds selected from the group consisting of:

- Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = SPh$, $R = H$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = SPh$, $R = H$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
 20 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = OPh$, $R = Ac$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = OPh$, $R = H$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = SCH_2CH_3$, $R = Ac$, $B = -\alpha Abu-$, and
 25 $U = -(D)Ala-$;
 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = SCH_2CH_3$, $R = H$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = OCH_2Ph$, $R = Ac$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
 30 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = OCH_2Ph$, $R = H$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = O\text{-}ortho\text{chlorophenyl}$, $R = Ac$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = O\text{-}ortho\text{chlorophenyl}$, $R = H$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
 35 $\alpha Abu-$, and $U = -(D)Ala-$;

Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = O\text{-pyridyl}$, $R = \text{Ac}$, $B = -\alpha\text{Abu-}$, and $U = -(D)\text{Ala-}$;

Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = O\text{-pyridyl}$, $R = \text{H}$, $B = -\alpha\text{Abu-}$, and $U = -(D)\text{Ala-}$;

- 5 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = S\text{-orthomethylphenyl}$, $R = \text{Ac}$, $B = -\alpha\text{Abu-}$, and $U = -(D)\text{Ala-}$;

Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = \text{SCN}$, $R = \text{Ac}$, $B = -\alpha\text{Abu-}$, and $U = -(D)\text{Ala-}$;

- 10 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = \text{NCO}$, $R = \text{Ac}$, $B = -\alpha\text{Abu-}$, and $U = -(D)\text{Ala-}$;

Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = \text{NHPh}$, $R = \text{Ac}$, $B = -\alpha\text{Abu-}$, and $U = -(D)\text{Ala-}$;

Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = \text{N(CH}_3\text{)Ph}$, $R = \text{Ac}$, $B = -\alpha\text{Abu-}$, and $U = -(D)\text{Ala-}$;

- 15 Compound of Formula (I): (A): $X = -CH_2\text{-CH=CH-(CH}_2)_2-$, $Y = \text{OH}$, $R = \text{Ac}$, $B = -\alpha\text{Abu-}$, and $U = -(D)\text{Ala-}$; and

Compound of Formula (I): (A): $X = -CH_2\text{-CH=CH-(CH}_2)_2-$, $Y = \text{OAc}$, $R = \text{Ac}$, $B = -\alpha\text{Abu-}$, and $U = -(D)\text{Ala-}$.

- 20 An alternate embodiment of the present invention is:

More particularly, the present invention provides a cyclosporin of the following Formula (I),

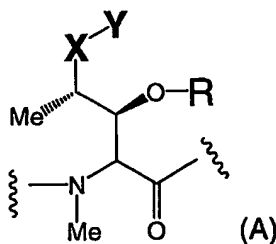


25

(I)

or a pharmaceutically acceptable salt, ester or prodrug thereof.

In Formula (I), A is



30

X is selected from the group consisting of: $-(CH_2)_n-$ and $-CH_2-CH=CH-(CH_2)_m-$,
 where n is an integer of from 3 to 8 and m is an integer of from 2 to 5;

Y is selected from the group consisting of:

deuterium;

5 halogen;

SCN;

NCO;

NCS;

OR₁, where R₁ is selected from the group consisting of:

10 C₁-C₆ alkyl, substituted with aryl, substituted aryl, heteroaryl, substituted
 heteroaryl, halogen,

C₃-C₆ alkenyl, substituted with aryl, substituted aryl, heteroaryl,
 substituted heteroaryl, halogen,

15 C₃-C₆ alkynyl, substituted with aryl, substituted aryl, heteroaryl,
 substituted heteroaryl, halogen,

aryl,

substituted aryl,

heteroaryl, and

substituted heteroaryl;

20 OC(O)-W-R₁, wherein W is absent, -O-, or -NH-, and R₁ is as previously
 defined;

OCH₂SR₂, wherein R₂ is C₁-C₆ alkyl optionally substituted with aryl,
 substituted aryl, heteroaryl, substituted heteroaryl;

NHR₁, wherein R₁ is as previously defined;

25 N(R₁)₂, wherein R₁ is as previously defined;

SR₃, wherein R₃ is selected from the group consisting of: thiol protecting
 group and R₁ as previously defined; and

S(O)_nR₁, where n is an integer 1 or 2 and R₁ is as previously defined;

30 R is selected from the group consisting of: hydrogen and a hydroxyl protecting
 group;

B is selected from the group consisting of: -αAbu-, -Val-, -Thr- and -Nva-; and

U is selected from the group consisting of: -(D)Ala-, -(D)Ser-, -[O-(2
 hydroxyethyl)(D)Ser]-, -[O-acyl(D)Ser]- and -[O-(2-acyloxyethyl)(D)Ser]-.

The potent immunomodulatory activity which compounds of the instant
5 invention demonstrate in common *in vitro* biological assays (for example, calcineurin phosphatase and binding assays, NFAT reporter gene assay, murine and human mixed lymphocyte reaction) or animal models (for example delayed-type hypersensitivity response - DTH, -allergen induced pulmonary eosinophilia) indicate that these compounds possess immunosuppressive, antimicrobial,
10 antifungal, antiviral, antiinflammatory, and antiproliferative activity, and possess the ability to reverse chemotherapeutic drug resistance. As agents block T-cell activation, a prerequisite for HIV proliferation, the compounds are useful as prophylactics for the prevention of HIV replication. The compounds of the invention would be useful when used alone, or in combination therapy with other
15 immunosuppressants, for example, but not limited to, FK506, rapamycin, cyclosporin A, picibanil, mycophenolic acid, azathioprine, prednisolone, cyclophosphamide, brequinar and leflunomide.

As immunosuppressants, the compounds of the present invention are useful
20 when administered for the prevention of immune-mediated tissue or organ graft rejection. Examples of transplanted tissues and organs which suffer from these effects are heart, kidney, liver, medulla ossium, skin, cornea, lung, pancreas, intestinum tenue, limb, muscle, nervus, duodenum, small-bowel, pancreatic-islet-cell, and the like; as well as graft-versus-host diseases brought about by medulla
25 ossium transplantation. The regulation of the immune response by the compounds of the invention would also find utility in the treatment of autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, hyperimmunoglobulin E, Hashimoto's thyroiditis, multiple sclerosis, progressive systemic sclerosis, myasthenia gravis, type I diabetes, uveitis, allergic encephalomyelitis,
30 glomerulonephritis, and the like; and further infectious diseases caused by pathogenic microorganisms, such as HIV. In the particular cases of HIV-1, HIV-2 and related retroviral strains, inhibition of T-cell mitosis would suppress the replication of the virus, since the virus relies upon the host T-cell's proliferative functions to replicate.

Further uses include the treatment and prophylaxis of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses, such as psoriasis, atopic dermatitis, contact dermatitis and
5 further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise) such as keratoconjunctivitis, vernal conjunctivitis, keratitis, herpetic keratitis, conical cornea,
10 dystrophia epithelialis corneae, corneal leukoma, ocular pemphigus, Mooren's ulcer, Scleritis, Graves' ophthalmopathy, Vogt-Koyanagi-Harada syndrome, sarcoidosis, multiple myeloma, etc.; obstructive airway diseases, which includes conditions such as chronic obstructive pulmonary disease (COPD), asthma (for example, bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and
15 dust asthma), particularly chronic or inveterate asthma (for example, late asthma and airway hyper-responsiveness), bronchitis, allergic rhinitis and the like; inflammation of mucosa and blood vessels such as gastric ulcers, vascular damage caused by ischemic diseases and thrombosis. Moreover, hyperproliferative vascular diseases such as intimal smooth muscle cell
20 hyperplasia, restenosis and vascular occlusion, particularly following biologically- or mechanically-mediated vascular injury can be treated or prevented by the compounds of the invention.

Other treatable conditions would include but are not limited to ischemic
25 bowel diseases, inflammatory bowel diseases, necrotizing enterocolitis, intestinal lesions associated with thermal burns and leukotriene B₄-mediated diseases; intestinal inflammations/allergies such as Coeliac diseases, proctitis, eosinophilic gastroenteritis, mastocytosis, Crohn's disease and ulcerative colitis; food-related allergic diseases which have symptomatic manifestation remote from the gastro-
30 intestinal tract (e.g., migraine, rhinitis and eczema); renal diseases such as interstitial nephritis, Goodpasture's syndrome, hemolytic-uremic syndrome and diabetic nephropathy; nervous diseases such as multiple myositis, Guillain-Barre syndrome, Meniere's disease, polyneuritis, multiple neuritis, mononeuritis and radiculopathy; endocrine diseases such as hyperthyroidism and Basedow's

disease; hematic diseases such as pure red cell aplasia, aplastic anemia, hypoplastic anemia, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, agranulocytosis, pernicious anemia, megaloblastic anemia and anerythroplasia; bone diseases such as osteoporosis; respiratory diseases such as sarcoidosis, fibroid lung and idiopathic interstitial pneumonia; skin disease such as dermatomyositis, leukoderma vulgaris, ichthyosis vulgaris, photoallergic sensitivity and cutaneous T cell lymphoma; circulatory diseases such as arteriosclerosis, atherosclerosis, aortitis syndrome, polyarteritis nodosa and myocardosis; collagen diseases such as scleroderma, Wegener's granuloma and Sjogren's syndrome; adiposis; eosinophilic fasciitis; periodontal disease such as lesions of gingiva, periodontium, alveolar bone and substantia ossea dentis; nephrotic syndrome such as glomerulonephritis; male pattern alopecia or alopecia senilis by preventing epilation or providing hair germination and/or promoting hair generation and hair growth; muscular dystrophy; Pyoderma and Sezary's syndrome; Addison's disease; active oxygen-mediated diseases, as for example organ injury such as ischemia-reperfusion injury of organs (such as heart, liver, kidney and digestive tract) which occurs upon preservation, transplantation or ischemic disease (for example, thrombosis and cardiac infraction); intestinal diseases such as endotoxin-shock, pseudomembranous colitis and colitis caused by drug or radiation; renal diseases such as ischemic acute renal insufficiency and chronic renal insufficiency; pulmonary diseases such as toxinoses caused by lung-oxygen or drug (for example, paracort and bleomycins), lung cancer and pulmonary emphysema; ocular diseases such as cataracta, siderosis, retinitis, pigmentosa, senile macular degeneration, vitreal scarring and corneal alkali burn; dermatitis such as erythema multiforme, linear IgA bullous dermatitis and cement dermatitis; and others such as gingivitis, periodontitis, sepsis, pancreatitis, diseases caused by environmental pollution (for example, air pollution), aging, carcinogenesis, metastasis of carcinoma and hypobaropathy; disease caused by histamine or leukotriene-C₄ release; Behcet's disease such as intestinal-, vasculo- or neuro-Behcet's disease, and also Behcet's which affects the oral cavity, skin, eye, vulva, articulation, epididymis, lung, kidney and so on.

Furthermore, the compounds of the invention are useful for the treatment and prevention of hepatic disease such as immunogenic diseases (for example, chronic autoimmune liver diseases such as the group consisting of autoimmune hepatitis, primary biliary cirrhosis and sclerosing cholangitis), partial liver resection, acute liver necrosis (e.g., necrosis caused by toxin, viral hepatitis, shock or anoxia), B-virus hepatitis, non-A/non-B hepatitis, cirrhosis (such as alcoholic cirrhosis) and hepatic failure such as fulminant hepatic failure, late-onset hepatic failure and "acute-on-chronic" liver failure (acute liver failure on chronic liver diseases), and moreover are useful for various diseases because of their useful activity such as augmentation of chemotherapeutic effect, preventing or treating activity of cytomegalovirus infection, particularly HCMV infection, anti-inflammatory activity, and so on.

The compounds of the present invention may be used as vaccines to treat immunosuppression in a subject. It is sometimes found that the antigen introduced into the body for the acquisition of immunity from disease also acts as an immunosuppressive agent, and therefore, antibodies are not produced by the body and immunity is not acquired. By introducing a compound of the present invention into the body as a vaccine, the undesired immunosuppression may be overcome and immunity acquired.

The compounds of the present invention may also find utility in the chemosensitization of drug resistant target cells. Cyclosporin A and FK-506 are known to be effective modulators of P-glycoprotein, a substance which binds to and inhibits the action of anticancer drugs by inhibiting P-glycoprotein, as they are capable of increasing the sensitivity of multidrug resistant (MDR) cells to chemotherapeutic agents. It is believed that the compounds of the invention may likewise be effective at overcoming resistance expressed to clinically useful antitumour drugs such as 5-fluorouracil, cisplatin, methotrexate, vincristine, vinblastine and adriamycin, colchicine and vincristine.

Further, it has recently been shown that the steroid receptor-associated heat shock proteins, hsp56 or hsp59, belong to the class of immunophilin proteins (*see* "HSP70 induction by cyclosporin A in cultured rat hepatocytes: effect of vitamin E

succinate," Andres, David et al., *Instituto de Bioqimica, Facultad de Farmacia, Universidad Complutense, Madrid, Spain*. J. Hepatol. (2000) 33(4), 570-579;
"Cyclosporin A Induces an Atypical Heat Shock Response," Paslaru, Liliana, et al.,
Unite de Genetique Moleculaire, Paris, Fr. Biochem. Biophys. Res. Commun.
5 (2000), 269(2), 464-469; "The cyclosporine A -binding immunophilin CyP-40 and
the FK506-binding immunophilin hsp56 bind to a common site on hsp90 and exist
in independent cytosolic heterocomplexes with the untransformed glucocorticoid
receptor," Owens-Grillo, Janet K. et al., Med. Sch., Univ. Michigan, Ann Arbor, MI
USA. J. Biol. Chem. (1995), 270(35), 20479-84). The ability of a steroid receptor-
10 associated heat shock protein to bind the immunosuppressive CsA suggests that
the steroid receptor and immunophilin signal transduction pathways are
functionally interrelated. The combined treatment of compounds of the present
invention and low concentrations of a steroid ligand (for e.g., progesterone,
dexamethasone) result in a significant enhancement of target gene expression
15 over that seen in response to ligand alone. Thus, the compounds of the present
invention potentiate steroid-mediated transactivation.

Aqueous liquid compositions of the present invention may be particularly
useful for the treatment and prevention of various diseases of the eye such as
20 autoimmune diseases (including, for example, conical cornea, keratitis, dysophia
epithelialis corneae, leukoma, Mooren's ulcer, scleritis and Graves'
ophthalmopathy) and rejection of corneal transplantation.

Accordingly, the pharmaceutical compositions of the present invention
25 comprise a therapeutically effective amount of a cyclosporin analog of the invention
in combination with a pharmaceutically acceptable carrier or excipient. In
particular, compositions pertaining to the present invention are useful for treating a
subject for immune-mediated organ or tissue allograft rejection, a graft-versus-host
disease, an autoimmune disease, an obstructive airway disease, a
30 hyperproliferative disease, or an ischemic or inflammatory intestinal or bowel
disease.

The present invention also relates to method(s) of treatment of immune
disorders and inflammation or prevention of organ transplant rejection in a subject

by administering to the subject therapeutically effective amounts of the cyclosporin analogs of the present invention with or without the concurrent use of other drugs or pharmaceutically acceptable excipients, as described throughout the present specification.

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The methods of the present invention comprise treating a subject in need of immunosuppressive, anti-inflammatory, antimicrobial, antifungal, antiviral or antiproliferative therapy, or requiring the reversal of chemotherapeutic drug resistance, by administering a therapeutically effective amount of a compound of the invention for such time and in such amounts as is necessary to produce the desired result.

10

As used in the present invention, "therapeutically effective amount" of one of the compounds means a sufficient amount of the compound to treat a particular disease, at a reasonable benefit/ risk ratio. The compounds of the present invention may be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester or prodrug forms. Alternatively, the compounds may be administered as pharmaceutical compositions containing the compound of interest in combination with one or more drugs or pharmaceutically acceptable excipients. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment.

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The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

30

The total daily dose of the compounds of this invention administered to a human or lower animal may range from about 0.001 to about 10 mg/kg of a patient's body mass/day. For purposes of oral administration, more preferable doses may be in the range of from about 0.005 to about 3 mg/kg/day. If desired, the effective daily dose may be divided into multiple doses for purposes of administration; consequently, single dose compositions may contain such amounts or submultiples thereof to make up the daily dose.

10 Definitions

The term "C₁-C₆-alkyl" as used herein refer to saturated, straight- or branched-chain hydrocarbon radicals containing between one and three or one and six carbon atoms, respectively. Examples of C₁-C₃-alkyl radicals include methyl, ethyl, propyl and isopropyl, and examples of C₁-C₆-alkyl radicals include, but are not limited to, methyl, ethyl, propyl, isopropyl, *n*-butyl, *tert*-butyl, neopentyl and *n*-hexyl.

The term "C₃-C₆ alkenyl" as used herein, denote a monovalent group derived from a hydrocarbon moiety containing from three to six carbon atoms having at least one carbon-carbon double bond by the removal of a single hydrogen atom. Alkenyl groups include, but are not limited to, for example, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, and the like.

The term "C₃-C₆ alkynyl" as used herein, denote a monovalent group derived from a hydrocarbon moiety containing from three to six carbon atoms having at least one carbon-carbon triple bond by the removal of a single hydrogen atom. Representative alkynyl groups include, but are not limited to, for example, ethynyl, 1-propynyl, 1-butyne, and the like.

The term "aryl," as used herein, refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, idenyl and the like. Aryl groups (including bicyclic aryl groups) can be unsubstituted or substituted with one, two or three

substitutents independently selected from loweralkyl, substituted loweralkyl, haloalkyl, alkoxy, thioalkoxy, amino, alkylamino, dialkylamino, acylamino, cyano, hydroxy, halo, mercapto, nitro, carboxaldehyde, carboxy, alkoxycarbonyl and carboxamide. In addition, substituted aryl groups include tetrafluorophenyl and pentafluorophenyl.

The term "substituted aryl," as used herein, refers to an aryl group, as defined herein, substituted by independent replacement of one, two or three of the hydrogen atoms thereon with F, Cl, Br, I, OH, NO₂, CN, C(O)-C₁-C₆-alkyl, C(O)-aryl, C(O)-heteroaryl, CO₂-alkyl, CO₂-aryl, CO₂-heteroaryl, CONH₂, CONH-C₁-C₆-alkyl, CONH-aryl, CONH-heteroaryl, OC(O)-C₁-C₆-alkyl, OC(O)-aryl, OC(O)-heteroaryl, OCO₂-alkyl, OCO₂-aryl, OCO₂-heteroaryl, OCONH₂, OCONH-C₁-C₆-alkyl, OCONH-aryl, OCONH-heteroaryl, NHC(O)-C₁-C₆-alkyl, NHC(O)-aryl, NHC(O)-heteroaryl, NHCO₂-alkyl, NHCO₂-aryl, NHCO₂-heteroaryl, NHCONH₂, NHCONH-C₁-C₆-alkyl, NHCONH-aryl, NHCONH-heteroaryl, SO₂-C₁-C₆-alkyl, SO₂-aryl, SO₂-heteroaryl, SO₂NH₂, SO₂NH-C₁-C₆-alkyl, SO₂NH-aryl, SO₂NH-heteroaryl, C₁-C₆-alkyl, C₃-C₆-cycloalkyl, CF₃, CH₂CF₃, CHCl₂, CH₂OH, CH₂CH₂OH, CH₂NH₂, CH₂SO₂CH₃, aryl, heteroaryl, benzyl, benzyloxy, aryloxy, heteroaryloxy, C₁-C₆-alkoxy, methoxymethoxy, methoxyethoxy, amino, benzylamino, arylamino, heteroarylamino, C₁-C₃-alkylamino, thio, aryl-thio, heteroarylthio, benzyl-thio, C₁-C₆-alkyl-thio, or methylthiomethyl.

The term "heteroaryl," as used herein, refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S, O and N; zero, one or two ring atoms are additional heteroatoms independently selected from S, O and N; and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms, such as, for example, pyridinyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

The term "substituted heteroaryl," as used herein, refers to a heteroaryl group as defined herein, substituted by independent replacement of one, two or

three of the hydrogen atoms thereon with F, Cl, Br, I, OH, NO₂, CN, C(O)-C₁-C₆-alkyl, C(O)-aryl, C(O)-heteroaryl, CO₂-alkyl, CO₂-aryl, CO₂-heteroaryl, CONH₂, CONH-C₁-C₆-alkyl, CONH-aryl, CONH-heteroaryl, OC(O)-C₁-C₆-alkyl, OC(O)-aryl, OC(O)-heteroaryl, OCO₂-alkyl, OCO₂-aryl, OCO₂-heteroaryl, OCONH₂, OCONH-C₁-C₆-alkyl, OCONH-aryl, OCONH-heteroaryl, NHC(O)-C₁-C₆-alkyl, NHC(O)-aryl, NHC(O)-heteroaryl, NHCO₂-alkyl, NHCO₂-aryl, NHCO₂-heteroaryl, NHCONH₂, NHCONH-C₁-C₆-alkyl, NHCONH-aryl, NHCONH-heteroaryl, SO₂-C₁-C₆-alkyl, SO₂-aryl, SO₂-heteroaryl, SO₂NH₂, SO₂NH-C₁-C₆-alkyl, SO₂NH-aryl, SO₂NH-heteroaryl, C₁-C₆-alkyl, C₃-C₆-cycloalkyl, CF₃, CH₂CF₃, CHCl₂, CH₂OH, CH₂CH₂OH, CH₂NH₂, CH₂SO₂CH₃, aryl, heteroaryl, benzyl, benzyloxy, aryloxy, heteroaryloxy, C₁-C₆-alkoxy, methoxymethoxy, methoxyethoxy, amino, benzylamino, arylamino, heteroarylamino, C₁-C₃-alkyl-amino, thio, aryl-thio, heteroarylthio, benzyl-thio, C₁-C₆-alkyl-thio, or methylthiomethyl.

The term "hydroxy protecting group," as used herein, refers to an easily removable group to which are known in the art to protect a hydroxyl group against undesirable reaction during synthetic procedures and to be selectively removable. The use of hydroxy-protecting groups is well known in the art for protecting groups against undesirable reactions during a synthetic procedure and many such protecting groups are known, *cf.* for example, T.H. Greene and P.G. M. Wuts, Protective Groups in Organic Synthesis, 3rd edition, John Wiley & Sons, New York (1999). Examples of hydroxy protecting groups include, but are not limited to, methylthiomethyl, *tert*-dimethylsilyl, *tert*-butyldiphenylsilyl, acyl substituted with an aromatic group and the like.

The term "thiol protecting group," as used herein, refers to an easily removable group to which are known in the art to protect a thiol group against undesirable reaction during synthetic procedures and to be selectively removable. The use of thiol-protecting groups is well known in the art for protecting groups against undesirable reactions during a synthetic procedure and many such protecting groups are known, *cf.* for example, T.H. Greene and P.G. M. Wuts, Protective Groups in Organic Synthesis, 3rd edition, John Wiley & Sons, New York

(1999). Examples of thiol protecting groups include, but are not limited to, acetyl, benzyl, p-methoxybenzyl and the like.

5 The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine and iodine.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, *et al.* describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977), which is incorporated herein by reference. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions

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such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

As used herein, the term "pharmaceutically acceptable salt" refers to those
5 salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, *et al.* describe pharmaceutically acceptable salts in detail in
10 J. Pharmaceutical Sciences, 66: 1-19 (1977), which is incorporated herein by reference. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with
15 inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate,
20 benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-
25 naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like.
30 Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

As used herein, the term "pharmaceutically acceptable ester" refers to esters which hydrolyze in vivo and include those that break down readily in the human body to leave the parent compound or a salt thereof. Suitable ester groups include, but are not limited to, those derived from pharmaceutically acceptable
5 aliphatic carboxylic acids, particularly alkanolic, alkenolic, cycloalkanoic and alkanedioic acids, in which each alkyl or alkenyl moiety advantageously has not more than 6 carbon atoms. Examples of particular esters include, but are not limited to, formates, acetates, propionates, butylates, acrylates and ethylsuccinates.

10

The term "pharmaceutically acceptable prodrugs" as used herein refers to those prodrugs of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and
15 the like, commensurate with a reasonable risk/reward ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. The term "prodrug" refers to compounds that are rapidly transformed in vivo to yield the parent compound of the above formulae, for example, by hydrolysis in blood. A thorough discussion is provided in T. Higuchi and V. Stella, Prodrugs as Novel delivery Systems, Vol. 14 of the A.C.S.
20 Symposium Series and in Edward B. Roche, ed., Bioreversible Carriers in Drug Design, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated by reference herein.

25

Abbreviations

	Sar:	Sarcosin
	Ac:	Acetyl
30	MeLeu:	N-Methyl-Leucine
	Val:	Valine
	Ala:	Alanine
	MeVal:	N-Methyl Valine
	Et:	Ethyl
35	Ph:	Phenyl

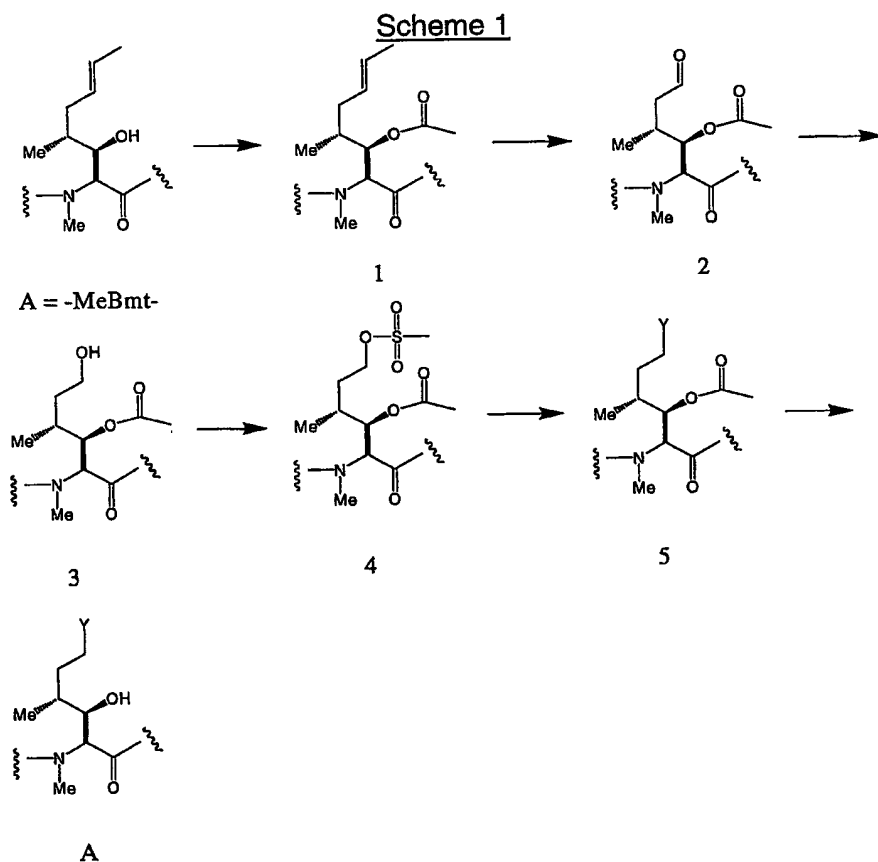
MeBmt: N-Methyl-butenyl-threonine

Synthetic Methods

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The compounds and processes of the present invention will be better understood in the following synthetic scheme which illustrates the methods by which the compounds of the present invention may be prepared. The groups B and U in Formula I are as defined above. A is -MeBmt- in the starting material as

10 illustrated in Scheme 1:



The process for the invention for the preparation of the compounds of Formula I comprises reacting cyclosporin A, a commercially available fermentation product wherein A = -MeBmt- with acetic anhydride, optionally in the presence of pyridine or dimethylaminopyridine, in dichloromethane to give acetylated cyclosporin A intermediate 1 (see Eberle, M. K., Nuninger, F. J. Org. Chem. 1992, 57, 2689-2691). Ozonolysis of intermediate 1 carried out at -78°C in

dichloromethane, followed by quenching with dimethylsulfide gives the aldehyde 2 (see Park, S. B., Meier, G. P. *Tetrahedron Lett.* 1989, 30, 4215-4218). Reduction of the aldehyde Intermediate 2 at 0°C with sodium borohydride gives the alcohol 3 (see Toshima, U., Tatsuta, K., Kinoshito, M. *Bull. Chem. Soc. Jpn* 1988, 61, 2369; Colombo, L., Di Giacomo, M. *Tetrahedron Lett.* 1999, 40, 1977), which is reacted with methanesulfonyl chloride and triethylamine in dichloromethane to give intermediate 4 (see Kitahara, T., Matsuoka, T., Katayama, M., Maramo, S., Mori, K. *Tetrahedron Lett.* 1984, 25, 4685; Ireland, R. E., Anderson, R. C., Badoud, R., Fitzsimmons, B. J. *J. Am. Chem. Soc.* 1983, 105, 1988). Intermediate 4 can be converted to Intermediate 5 by displacement with a nucleophile, such as, but not limited to sodium azide, sodium phenoxide, sodium thiophenoxide, sodium cyanide in dimethylformamide or tetrahydrofuran at room temperature to 60°C for 3 to 48 hours (see Effenberger, F., Stelzer, U. *Angew. Chem.* 1991, 103, 866; Fleming, P. R., Sharpless, K. B. *J. Org. Chem.* 1991, 56, 2869). Intermediate 5 is then converted to the compound of Formula I by hydrolysis with potassium carbonate in methanol (see Plattner, J. J., Gless, R. D., Rapoport, H. J. *Am. Chem. Soc.* 1972, 94, 8613).

Pharmaceutical Compositions

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In the pharmaceutical compositions of the present invention, a compound of the invention is combined with a pharmaceutically acceptable carrier or excipient, meaning a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or Formulation auxiliary of any type. The compositions may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

30

Pharmaceutical compositions of this invention for parenteral injection comprise pharmaceutically-acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of

suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper
5 fluidity may be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservative,
10 wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable
15 pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of the drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This
20 may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

25

Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide, poly(orthoesters) and poly(anhydrides). Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release
30 can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

The injectable formulations may be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form

of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Solid dosage forms for oral administration include capsules, tablets, pills,
5 powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically-acceptable excipient or carrier, such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone,
10 sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h)
15 absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

20 Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules
25 may be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can
30 be used include polymeric substances and waxes.

The active compounds can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include pharmaceutically-acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Suspensions may contain, in addition to the active compounds, suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, and tragacanth, and mixtures thereof.

Topical administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for topical administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized. In non-pressurized powder compositions, the active ingredient in finely divided form may be used in admixture with a larger-sized pharmaceutically-acceptable inert carrier comprising particles having a size, for example, of up to 100 micrometers in diameter. Suitable inert carriers include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 micrometers.

Alternatively, the composition may be pressurized and contain a compressed gas, such as nitrogen or a liquified gas propellant. The liquified propellant medium and indeed the total composition are preferably such that the active ingredient does not dissolve therein to any substantial extent. The

pressurized composition may also contain a surface-active agent, such as a liquid or solid non-ionic surface-active agent or may be a solid anionic surface-active agent. It is preferred to use the solid anionic surface-active agent in the form of a sodium salt.

5

A further form of topical administration is to the eye, as for the treatment of immune-mediated conditions of the eye such as autoimmune diseases, allergic or inflammatory conditions, and corneal transplants. The compound of the invention is delivered in a pharmaceutically acceptable ophthalmic vehicle, such that the compound is maintained in contact with the ocular surface for a sufficient time period to allow the compound to penetrate the corneal and internal regions of the eye, as for example the anterior chamber, posterior chamber, vitreous body, aqueous humor, vitreous humor, cornea, iris/ciliary, lens, choroid/retina and sclera. The pharmaceutically acceptable ophthalmic vehicle may, for example, be an ointment, vegetable oil or an encapsulating material.

15

Compositions for rectal or vaginal administration are preferably suppositories which may be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at room temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

20

Compounds of the present invention may also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 *et seq.*

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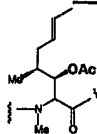
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Examples

The procedures described above for preparing the compounds of the present invention will be better understood in connection with the following examples, which are intended to be illustrative only and not limiting of the scope of the invention. Various changes and modifications of the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation, those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, formulations and/or methods for the invention may be made without departing from the spirit of the invention and the scope of the appended claims.

15

Example 1.

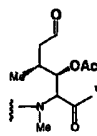



Compound of Formula (I): A = , B = $-\alpha\text{Abu}-$ and U = $-(\text{D})\text{Ala}-$.

To a solution of cyclosporin A (20 g, 16.6 mmol) in methylene chloride (40 mL) were added pyridine (10.07 mL, 124.5 mmol), dimethylaminopyridine (2.03 g, 16.6 mmol) and acetic anhydride (7.83 mL, 83 mmol) dropwise. The reaction mixture was stirred at ambient temperature for 18 hours. Subsequently, the mixture was diluted with ethyl acetate and washed with 1N HCl, 1M NaOH and brine. The organic layer was dried over magnesium sulfate and concentrated *in vacuo* to give the title compound as a white solid (20.7 g, 100% yield). Electropray mass spectrum (ESMS) $M+H$: 1244.48.

25

Example 2.



Compound of Formula (I): A = , B = $-\alpha\text{Abu}-$ and U = $-(\text{D})\text{Ala}-$.

30

A solution of the cyclosporin acetate-protected derivative from Example 1 (20.7 g, 16.6 mmol) in methylene chloride (40 mL) was cooled to -78°C with a dry ice/acetone bath and ozone was bubbled through the solution until the blue color persisted. Subsequently, oxygen was bubbled through the reaction mixture for 15 minutes and the reaction was quenched with dimethylsulfide (4 mL) and allowed to warm to ambient temperature overnight. The solution was then concentrated *in vacuo* to afford the title compound as a clear oil (20.5 g, 100% yield).

ESMS M+H: 1232.31.

10

Example 3.

Compound of Formula (I): (A): X = -(CH₂)₂-, Y = OH, R = Ac, B = -αAbu- and U = -(D)Ala-

15

A solution of the cyclosporin acetate-protected aldehyde derivative from Example 2 (20.5 g, 16.6 mmol) in anhydrous methanol (30 mL) was cooled to below 0°C with a water/brine bath and sodium borohydride (6.28 g, 166 mmol) was added slowly over 30 minutes. After 1 hour, the reaction was quenched with water and 1 N HCl and diluted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate and concentrated *in vacuo* to give the title compound as a white solid (16.26g, 79% yield).

20

ESMS M+H: 1234.37.

Example 4.

Compound of Formula (I): (A): X = -(CH₂)₂-, Y = OS(O)₂CH₃, R = Ac, B = -αAbu- and U = -(D)Ala-

25

To a cold (0°C) solution of the cyclosporin acetate-protected alcohol derivative from Example 3 (15.33 g, 12.42 mmol) in methylene chloride (200 ml) was added methanesulfonyl chloride (1.44 mL, 18.63 mmol) and triethylamine (5.2 mL, 37.26 mmol) and the reaction was stirred at 0°C for 3 hours and then stored at 4°C for 18 hours. Subsequently, the reaction was diluted with ethyl acetate washed with saturated sodium bicarbonate, brine and dried over magnesium sulfate. After concentration *in vacuo*, the title compound was obtained as an orange solid (14.63 g, 90% yield).

30

ESMS M+H: 1312.53.

Example 5

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = SPh, R=Ac, B = $-\alpha Abu-$, and U = $-(D)Ala-$.

- 5 To a cold (0°C) solution of thiophenol (0.157 ml, 1.53 mmol) in anhydrous tetrahydrofuran (3 ml) was added sodium hydride (0.052 mg, 1.38 mmol, 60% suspension in oil) and the reaction was stirred at 0°C for 20 min. Afterwards, the title compound of example 4 (0.2 g, 0.153 mmol) was added and the reaction was warmed up to ambient temperature over 18 h (or sometimes reflux over 18 h).
- 10 Afterwards, the mixture was diluted with ethyl acetate and water and the organic layer was washed with 1N HCl. Drying over magnesium sulfate and concentration in vacuo gave the product as a yellow solid (0.1 g, 49% yield). (ESMS) M+Na: 1348.21

Example 6

- 15 Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = SPh, R=H, B = $-\alpha Abu-$, and U = $-(D)Ala-$.

- A solution of potassium carbonate (0.052 g, 0.377 mmol) and the title compound of example 5 (0.1 g, 0.075 mmol) in anhydrous methanol (3 ml) was stirred at ambient temperature for 18 h. Afterwards, the reaction mixture was diluted with ethyl acetate and washed with 1N HCl and brine, dried over magnesium sulfate and concentrated in vacuo. Flash chromatography (silica gel, methanol/ether) gave the product as a yellow solid.
- 20 (ESMS) M+H: 1284.

25

Example 7

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = OPh, R=Ac, B = $-\alpha Abu-$, and U = $-(D)Ala-$.

- The title compound was prepared from acetate protected CsA mesylate from Example 4 and sodium phenoxide according to the procedures described in Example 5.
- 30 (ESMS) M+H: 1309.86.

Example 8

- 35 Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = OPh, R=H, B = $-\alpha Abu-$, and U = $-(D)Ala-$.

The title compound of was prepared from the title compound of Example 7 and potassium carbonate in methanol according to the procedures described in example 6.

(ESMS) M+H: 1267.85.

5

Example 9

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = SCH_2CH_3 , R=Ac, B = $-\alpha Abu-$, and U = $-(D)Ala-$.

The title compound of was prepared from acetate protected CsA mesylate from Example 4 and ethanethiol according to the procedures described in Example 5.

(ESMS) M+H: 1277.84.

Example 10

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = SCH_2CH_3 , R=H, B = $-\alpha Abu-$, and U = $-(D)Ala-$.

The title compound of was prepared from the title compound of Example 9 according to the procedures described in Example 6.

(ESMS) M+H: 1235.83.

20

Example 11

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = OCH_2Ph , R=Ac, B = $-\alpha Abu-$, and U = $-(D)Ala-$.

The title compound is prepared from the title compound of Example 4, benzyl alcohol and sodium hydride according to the procedures described in Example 5.

Example 12

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = OCH_2Ph , R=H, B = $-\alpha Abu-$, and U = $-(D)Ala-$.

The title compound is prepared from the title compound of Example 11 according to the procedures described in Example 6.

Example 13

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = O-orthochlorophenyl, R=Ac, B = $-\alpha Abu-$, and U = $-(D)Ala-$.

The title compound is prepared from the title compound from Example 4, ortho chlorophenol and sodium hydride according to the procedures described in Example 5.

5

Example 14

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = O-orthochlorophenyl, R=H, B = $-\alpha$ Abu-, and U = $-(D)Ala-$.

The title compound is prepared from the title compound of Example 13 according to the procedures described in Example 6.

10

Example 15

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = O-pyridyl, R=Ac, B = $-\alpha$ Abu-, and U = $-(D)Ala-$.

The title compound of is prepared from the title compound of Example 4 and ortho hydroxypyridine and sodium hydride according to the procedures described in Example 5.

15

Example 16

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = O-pyridyl, R=H, B = $-\alpha$ Abu-, and U = $-(D)Ala-$.

20

The title compound of Example 16 is prepared from the title compound of Example 15 according to the procedures described in Example 6.

Example 17

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = S-orthomethylphenyl, R=Ac, B = $-\alpha$ Abu-, and U = $-(D)Ala-$.

25

The title compound is prepared from the title compound of Example 4 and ortho methylthiophenol and sodium hydride according to the procedures described in Example 5.

30

Example 18

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = SCN, R=Ac, B = $-\alpha$ Abu-, and U = $-(D)Ala-$.

35

To a solution of KSCN (0.15 g, 1.53 mmol) in anhydrous dimethylformamide (2 ml) was added the title compound of example 4 (0.2 g, 0.153 mmol) and the reaction was heated at 60°C for 18 h. Subsequently, the reaction was diluted with ethyl acetate and washed with sodium bicarbonate and brine. Drying over

magnesium sulfate and concentration in vacuo afforded the product as an off-white solid (92% yield).

ESMS M+K: 1313.63.

5

Example 19

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = NCO, R=Ac, B = $-\alpha$ Abu-, and U = $-(D)Ala-$.

The title compound is prepared from the title compound of Example 4 and KNCO according to the procedures described in Example 18.

10

Example 20

Compound of Formula (I): (A): X = $-\text{CH}_2\text{-CH=CH-(CH}_2)_2-$, Y = OAc, R=Ac, B = $-\alpha$ Abu-, and U = $-(D)Ala-$.

The title compound is prepared by reacting cyclosporin A (1 eq.) with $\text{CH}_2=\text{CH-(CH}_2)_2\text{-OAc}$ (5 eq.) and 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene(tricyclohexylphosphine)dichloro ruthenium(II)benzylidene (0.1 eq.) in toluene at 80°C for 18 hours. After concentration *in vacuo* and flash chromatography (silica gel/ 2:1 hexane/acetone) affords the product.

20

Example 21

Compound of Formula (I): (A): X = $-\text{CH}_2\text{-CH=CH-(CH}_2)_2-$, Y = OH, R=Ac, B = $-\alpha$ Abu-, and U = $-(D)Ala-$.

The title compound is prepared by hydrolyzing the title compound from Example 20 and potassium carbonate in methanol according to the procedures described in Example 6.

25

Example 22

Compound of Formula (I): (A): X = $-(CH_2)_2-$, Y = $-\text{NHPh}$, R=Ac, B = $-\alpha$ Abu-, and U = $-(D)Ala-$.

30

The title compound is prepared from the title compound from Example 4, aniline and sodium hydride according to the procedures described in Example 5.

35

Example 23

Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = -N(CH_3)Ph$, $R = Ac$, $B = -\alpha Abu-$,
and $U = -(D)Ala-$.

The title compound is prepared from the title compound from Example 4, N-methylaniline and sodium hydride according to the procedures described in Example 5.

The cyclosporins of the present invention have potent immunosuppressive anti-inflammatory activity. In particular they inhibit antigen-induced inflammatory cell infiltration, for example, into the airways. *In vivo* this activity is apparent following topical administration, e.g., via the pulmonary route.

Anti-inflammatory properties of the cyclosporins of the invention may be demonstrated in standard test models *in vitro* and *in vivo*, e.g., as follows.

15

Example 24Calcineurin Inhibition Assay

The immunosuppressive activity of cyclosporin is mediated through inhibition of the phosphatase activity of the enzyme calcineurin by a cyclophilin-cyclosporin complex. Thus, calcineurin inhibition is widely used as an *in vitro* measure of the activity of cyclosporin analogs.

Compounds were tested in an assay based on the Biomol Green Calcineurin Assay Kit supplied by Biomol (Plymouth Meeting, PA), supplemented with cyclophilin A for enzyme inhibition. The activity of the recombinant human calcineurin was determined by release of phosphate from a phosphopeptide representing a fragment of camp-dependent protein kinase. Phosphate release was determined using the colorimetric detection reagent Biomol Green.

Compounds in DMSO (2.4 μ l) were added to a 96-well microplate and mixed with 50 μ l assay buffer (50 mM Tris, pH 7.5, 0.1 M sodium chloride, 6 mM magnesium chloride, 0.5 mM dithiothreitol, 0.025% NP-40, 0.5 mM calcium chloride, 0.25 μ M calmodulin) containing 5 μ M cyclophilin and 20 units of calcineurin. After warming to 37 °C for 15 min, the enzymatic reaction was initiated by addition of phosphopeptide (7.5 μ l) to give a final concentration of 94 μ M. Phosphate release after 60 min at 37 °C was determined by addition of Biomol

Green (100 μ l) and measurement of the absorbance at 620 nm after 15 min at room temperature.

IC₅₀ values were calculated from determinations of enzyme activity at inhibitor concentrations ranging from 20 to 0.006 μ M.

5

Example 25

Immunosuppressive Activity and Applications

Murine Mixed Lymphocyte Reaction

Approximately 0.5×10^6 lymphocytes from the spleen of female (8-10 weeks) Balb/c mice are incubated for 5 days in 0.2 ml cell growth medium with ca. 0.5×10^6 lymphocytes from the spleen of female (8-10 weeks) CBA mice. Test substance is added to the medium at various concentrations. Activity is assessed by ability to suppress proliferation-associated DNA synthesis as determined by incorporation of radiolabelled thymidine.

15

Mishell-Dutton Test

Approximately 10^7 lymphocytes from the spleen of OF1, female mice are co-cultured with ca. 3×10^7 sheep erythrocytes for 3 days. Test substance is added to the incubation medium in varying concentrations. Lymphocytes are harvested and plated onto agar with fresh sheep erythrocytes as antigen. Sensitized lymphocytes secrete antibody that coats the erythrocytes, which lyse to form a plaque in the presence of complement. Activity is assessed by reduction in the number of plaque forming, i.e., antibody product, cells.

20

Influence on Allergen-Induced Pulmonary Eosinophilia (*in vitro*)

Male Himalayan spotted guinea pigs (300 g, BRL) are sensitized to ovalbumin (OA) by i.p. injection of 1 ml of a suspension of OA (10 μ g/ml) with Al(OH)₃ (100 mg) and B-pertussis vaccine (0.25 ml) in saline (0.9% w/v). For oral studies the procedure is repeated 1x after 2 weeks and the animals are used one week later. For inhalation studies the procedure is repeated 2x at 3-week intervals and the animals are used one week after the last injection.

30

Challenge is affected employing a saline solution of OA, nebulized for discharge into an exposure chamber. Test animals are exposed to OA by nose-only inhalation for 60 minutes. For inhalation studies, OA solution is used at a concentration of 0.01%.

Test substance is administered by inhalation and/or orally. For oral studies, test substance is administered p.o. in olive oil 1x daily for 3 days or in powder form in methylcellulose once prior to OA challenge. On day 3, test animals receive test substance 1.5 hours prior to and 6 hours after OA challenge. For inhalation
5 studies, test substance is micronised for delivery to test animals restrained within a flow-past, nose-only inhalation chamber. Administration by inhalation is effected 15 minutes prior to OA challenge.

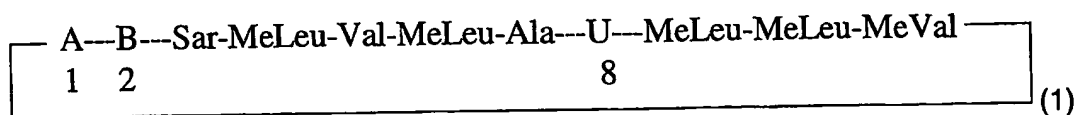
Efficacy of administered test substance is determined by bronchoalveolar lavage (BAL) and cell counting. For this purpose animals are sacrificed with Na
10 pento-barbitone (100 mg/kg i.p.) and the trachea is exposed and cannulated. 5 successive 10 ml aliquots of Ca^{2+} and Mg^{2+} free Hank's balanced salt solution (HBSS), containing bovine serum albumin (BSA, 0.3%), EDTA (10mM) and HEPES (10 mM) is then introduced into the lung and immediately aspirated by gentle compression of the lung tissue. Total cell counts in pooled eluates are
15 determined using an automatic cell counter. Lavage fluid is centrifuged at 200g for 10 minutes and the cell pellet resuspended in 1 ml of supplemented HBSS. 10 μl of this cell suspension is added to 190 μl of Turk's solution (1:20) dilution). Differential cell counts are made from smears stained by Diff-Quick. Cells are identified and counted under oil immersion (x1,000). A minimum of 500 cells per
20 smear are counted and the total population of each cell type is calculated.

Although the invention has been described with respect to various preferred embodiments, it is not intended to be limited thereto, but rather those skilled in the art will recognize that variations and modifications may be made therein which are
25 within the spirit of the invention and the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A compound of Formula (I)

5

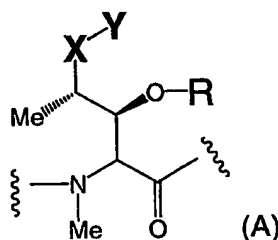


or a pharmaceutically acceptable salt, ester, or prodrug thereof:

wherein:

A is

10



X is selected from the group consisting of: $-(\text{CH}_2)_n-$ and $-\text{CH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_m-$,

where n is an integer of from 2 to 8 and m is an integer of from 2 to 5;

15 Y is selected from the group consisting of:

deuterium;

halogen;

SCN;

NCO;

20

NCS;

OR₁, where R₁ is selected from the group consisting of:

hydrogen,

C₁-C₆ alkyl optionally substituted with aryl, substituted aryl, heteroaryl,
substituted heteroaryl, halogen,

25

C₃-C₆ alkenyl optionally substituted with aryl, substituted aryl, heteroaryl,
substituted heteroaryl, halogen,

C₃-C₆ alkynyl optionally substituted with aryl, substituted aryl, heteroaryl,
substituted heteroaryl, halogen,

aryl,

30

substituted aryl,

- heteroaryl, and
substituted heteroaryl;
OC(O)-W-R₁, wherein W is absent, -O-, or -NH-, and R₁ is as previously
defined;
- 5 OCH₂SR₂, wherein R₂ is C₁-C₆ alkyl optionally substituted with aryl,
substituted aryl, heteroaryl, substituted heteroaryl;
NHR₁, wherein R₁ is as previously defined;
N(R₁₀)R₁, wherein R₁ is as previously defined and R₁₀ is selected from the
group consisting of: hydrogen, -CH₃, and -CH₂CH₃;
- 10 SR₃, wherein R₃ is selected from the group consisting of: hydrogen, thiol
protecting group, and R₁ as previously defined; and
S(O)_nR₁, where n is an integer 1 or 2 and R₁ is as previously defined;
R is selected from the group consisting of: hydrogen and a hydroxyl protecting
group;
- 15 B is selected from the group consisting of: -αAbu-, -Val-, -Thr- and -Nva-; and
U is selected from the group consisting of: -(D)Ala-, -(D)Ser-, -[O-(2
hydroxyethyl)(D)Ser]-, -[O-acyl(D)Ser]- and -[O-(2-acyloxyethyl)(D)Ser]-.

2. A compound according to claim 1 which is selected from the group
- 20 consisting of:
- Compound of Formula (I): (A): X = -(CH₂)₂-, Y = SPh, R=Ac, B = -αAbu-, and U =
-(D)Ala-;
- Compound of Formula (I): (A): X = -(CH₂)₂-, Y = SPh, R=H, B = -αAbu-, and U = -
(D)Ala-;
- 25 Compound of Formula (I): (A): X = -(CH₂)₂-, Y = OPh, R=Ac, B = -αAbu-, and U =
-(D)Ala-;
- Compound of Formula (I): (A): X = -(CH₂)₂-, Y = OPh, R=H, B = -αAbu-, and U = -
(D)Ala-;
- Compound of Formula (I): (A): X = -(CH₂)₂-, Y = SCH₂CH₃, R=Ac, B = -αAbu-, and
30 U = -(D)Ala-;
- Compound of Formula (I): (A): X = -(CH₂)₂-, Y = SCH₂CH₃, R=H, B = -αAbu-, and
U = -(D)Ala-;
- Compound of Formula (I): (A): X = -(CH₂)₂-, Y = OCH₂Ph, R=Ac, B = -αAbu-, and
U = -(D)Ala-;

- Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = OCH_2Ph$, $R=H$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
- Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = O$ -ortho-chlorophenyl, $R=Ac$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
- 5 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = O$ -ortho-chlorophenyl, $R=H$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
- Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = O$ -pyridyl, $R=Ac$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
- 10 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = O$ -pyridyl, $R=H$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
- Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = S$ -orthomethylphenyl, $R=Ac$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
- Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = SCN$, $R=Ac$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
- 15 Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = NCO$, $R=Ac$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
- Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = NHPh$, $R=Ac$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
- Compound of Formula (I): (A): $X = -(CH_2)_2-$, $Y = N(CH_3)Ph$, $R=Ac$, $B = -\alpha Abu-$, and $U = -(D)Ala-$;
- 20 Compound of Formula (I): (A): $X = -CH_2-CH=CH-(CH_2)_2-$, $Y = OH$, $R=Ac$, $B = -\alpha Abu-$, and $U = -(D)Ala-$; and
- Compound of Formula (I): (A): $X = -CH_2-CH=CH-(CH_2)_2-$, $Y = OAc$, $R=Ac$, $B = -\alpha Abu-$, and $U = -(D)Ala-$.

25

3. A pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) in claim 1, or a pharmaceutically acceptable salt, ester or prodrug thereof, in combination with a pharmaceutically acceptable carrier or excipient.

30

4. A method of treating organ transplantation rejection in a subject, which comprises administering to said subject a therapeutically effective amount of the pharmaceutical composition of claim 3.

5. A method of treating an immune disorder in a subject, which comprises administering to said subject a therapeutically effective amount of the pharmaceutical composition of claim 3.

5 6. The method of claim 5, wherein said immune disorder is selected from the group consisting of: rheumatoid arthritis, inflammatory bowel disease, psoriasis, asthma, atopic dermatitis, allergic rhinitis, and chronic obstructive pulmonary disease.

10 7. A method of treating an immune disorder in a subject, which comprises topically administering to said subject a therapeutically effective amount of the pharmaceutical composition of claim 3.

15 8. The method of claim 7, wherein said inflammatory or immune disorder is selected from the group consisting of psoriasis and eczema.

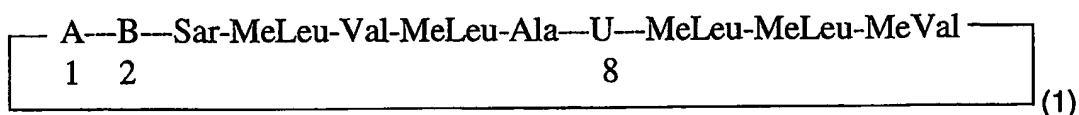
9. The method of claim 7, wherein said topically administering is achieved via inhalation.

20 10. The method of claim 9, wherein said inflammatory or immune disorder is an obstructive airways disease.

25 11. The method of claim 10, wherein said airways disease is selected from the group consisting of asthma, allergic rhinitis, bronchitis, cystic fibrosis, and chronic obstructive pulmonary disease.

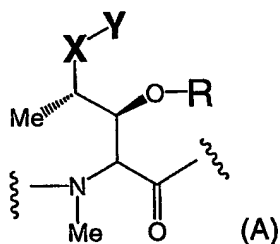
12. The method of claim 16, wherein said chronic obstructive pulmonary disease is emphysema or chronic bronchitis.

30 13. A compound of Formula (I):



or a pharmaceutically acceptable salt, ester, or prodrug thereof:

wherein A is :



- 5 X is selected from the group consisting of: $-(CH_2)_n-$ and $-CH_2-CH=CH-(CH_2)_m-$,
where n is an integer of from 3 to 8 and m is an integer of from 2 to 5;

Y is selected from the group consisting of:

deuterium;

SCN;

10 NCO;

NCS;

OR₁, where R₁ is selected from the group consisting of:

C₁-C₆ alkyl, substituted with aryl, substituted aryl, heteroaryl, substituted
heteroaryl, halogen,

15 C₃-C₆ alkenyl, substituted with aryl, substituted aryl, heteroaryl,
substituted heteroaryl, halogen,

C₃-C₆ alkynyl, substituted with aryl, substituted aryl, heteroaryl,
substituted heteroaryl, halogen,

aryl,

20 substituted aryl,

heteroaryl, and

substituted heteroaryl;

OC(O)-W-R₁, wherein W is absent, -O-, or -NH-, and R₁ is as previously
defined;

25 OCH₂SR₂, wherein R₂ is C₁-C₆ alkyl optionally substituted with aryl,
substituted aryl, heteroaryl, substituted heteroaryl;

NHR₁, wherein R₁ is as previously defined;

N(R₁)₂, wherein R₁ is as previously defined;

30 SR₃, wherein R₃ is selected from the group consisting of: thiol protecting
group and R₁ as previously defined; and

$S(O)_nR_1$, where n is an integer 1 or 2 and R_1 is as previously defined;
R is selected from the group consisting of: hydrogen and a hydroxyl protecting group;

B is selected from the group consisting of: $-\alpha\text{Abu-}$, $-\text{Val-}$, $-\text{Thr-}$ and $-\text{Nva-}$; and

5 U is selected from the group consisting of: $-(\text{D})\text{Ala-}$, $-(\text{D})\text{Ser-}$, $-\text{[O-(2-hydroxyethyl)(D)Ser]-}$, $-\text{[O-acyl(D)Ser]-}$ and $-\text{[O-(2-acyloxyethyl)(D)Ser]-}$.

14. A pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) in claim 13, or a pharmaceutically acceptable salt, ester or prodrug thereof, in combination with a pharmaceutically acceptable carrier or excipient.

15. A method of treating organ transplantation rejection in a subject, which comprises administering to said subject a therapeutically effective amount of the pharmaceutical composition of claim 14.

16. A method of treating an immune disorder in a subject, which comprises administering to said subject a therapeutically effective amount of the pharmaceutical composition of claim 14.

20 17. The method of claim 16, wherein said immune disorder is selected from the group consisting of: rheumatoid arthritis, inflammatory bowel disease, psoriasis, asthma, atopic dermatitis, allergic rhinitis, and chronic obstructive pulmonary disease.

25 18. A method of treating an immune disorder in a subject, which comprises topically administering to said subject a therapeutically effective amount of the pharmaceutical composition of claim 14.

30 19. The method of claim 18, wherein said inflammatory or immune disorder is selected from the group consisting of psoriasis and eczema.

20. The method of claim 18, wherein said topically administering is achieved via inhalation.

21. The method of claim 20, wherein said inflammatory or immune disorder is an obstructive airways disease.

5 22. The method of claim 21, wherein said airways disease is selected from the group consisting of asthma, allergic rhinitis, bronchitis, cystic fibrosis, and chronic obstructive pulmonary disease.

10 23. The method of claim 22, wherein said chronic obstructive pulmonary disease is emphysema or chronic bronchitis.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/003582

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K7/64 A61K38/13

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, EMBASE, CHEM ABS Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/069902 A (LAZAROVA TSVETELINA ; OR YAT SUN (US); ENANTA PHARMACEUTICALS INC (US)) 12 September 2002 (2002-09-12) the whole document	1-23
X	EP 0 577 544 A (SANDOZ LTD ; SANDOZ AG (DE)) 5 January 1994 (1994-01-05) the whole document	1, 3-23
A		2
A	US 4 554 351 A (WENGER ROLAND) 19 November 1985 (1985-11-19) the whole document	1-23
A	EP 0 283 801 A (ABBOTT LAB) 28 September 1988 (1988-09-28) the whole document	1-23
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *G* document member of the same patent family

Date of the actual completion of the international search

27 July 2004

Date of mailing of the international search report

05/08/2004

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Pinheiro Vieira, E

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/003582

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 03/033010 A (LAZAROVA TSVETELINA ; CHEN JASON (US); HAMANN BLAKE (US); OR YAT SUN () 24 April 2003 (2003-04-24) the whole document -----	1-23
P,X	WO 03/030834 A (LAZAROVA TSVETELINA ; OR YAT SUN (US); ENANTA PHARMACEUTICALS INC (US)) 17 April 2003 (2003-04-17) the whole document -----	1-23
E	WO 2004/050687 A (ENANTA PHARMACEUTICALS INC) 17 June 2004 (2004-06-17) the whole document -----	1-23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/003582

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 4-12 and 15-23 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US2004/003582

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 02069902	A	12-09-2002	US 2002142946 A1	03-10-2002
			CA 2439832 A1	12-09-2002
			EP 1365791 A2	03-12-2003
			WO 02069902 A2	12-09-2002
			US 2003109426 A1	12-06-2003
			US 2003186855 A1	02-10-2003
EP 0577544	A	05-01-1994	AT 146484 T	15-01-1997
			AU 3387293 A	09-09-1993
			CA 2090634 A1	03-09-1993
			CN 1076453 A ,B	22-09-1993
			CZ 9300303 A3	16-02-1994
			DE 69306670 D1	30-01-1997
			DE 69306670 T2	03-07-1997
			DK 577544 T3	01-04-1997
			EP 0577544 A1	05-01-1994
			ES 2096262 T3	01-03-1997
			FI 930912 A	03-09-1993
			GR 3022539 T3	31-05-1997
			HU 64567 A2	28-01-1994
			IL 104891 A	15-04-1997
			JP 3382656 B2	04-03-2003
			JP 7252296 A	03-10-1995
			KR 256988 B1	15-05-2000
			MX 9301129 A1	29-07-1994
			NO 930738 A	03-09-1993
			NZ 247031 A	26-05-1995
			PL 297902 A1	06-09-1993
			RU 2131883 C1	20-06-1999
			SK 14093 A3	06-10-1993
			US 5643870 A	01-07-1997
			ZA 9301491 A	02-09-1994
US 4554351	A	19-11-1985	AT 10188 T	15-11-1984
			AT 43335 T	15-06-1989
			AT 28744 T	15-08-1987
			AU 591455 B2	07-12-1989
			AU 4361085 A	14-11-1985
			AU 6729181 A	20-08-1981
			AU 548156 B2	28-11-1985
			AU 6729781 A	20-08-1981
			DE 3167014 D1	13-12-1984
			DE 3176340 D1	10-09-1987
			DE 3177056 D1	29-06-1989
			EP 0034567 A2	26-08-1981
			EP 0098456 A2	18-01-1984
			EP 0098457 A2	18-01-1984
			IE 54936 B1	28-03-1990
			JP 1667321 C	29-05-1992
			JP 3023560 B	29-03-1991
			JP 56128725 A	08-10-1981
			JP 1794247 C	14-10-1993
			JP 2000137 A	05-01-1990
			JP 4078629 B	11-12-1992
			US 4396542 A	02-08-1983
EP 0283801	A	28-09-1988	EP 0283801 A2	28-09-1988
			JP 2661948 B2	08-10-1997

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US2004/003582

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 0283801	A		JP 63258491 A	25-10-1988
			US 5427960 A	27-06-1995
			US 5239057 A	24-08-1993
WO 03033010	A	24-04-2003	US 2003104992 A1	05-06-2003
			EP 1434594 A1	07-07-2004
			WO 03033010 A1	24-04-2003
WO 03030834	A	17-04-2003	US 2003087813 A1	08-05-2003
			WO 03030834 A2	17-04-2003
			US 2003109425 A1	12-06-2003
			US 2003166515 A1	04-09-2003
WO 2004050687	A	17-06-2004	US 2004110666 A1	10-06-2004
			WO 2004050687 A2	17-06-2004